Review article

Mimicking cigarette smoke exposure to assess cutaneous toxicity

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Abstract:

Cigarette smoke stands among the most toxic environmental pollutants and is composed of thousands of chemicals including polycyclic aromatic hydrocarbons (PAHs). Despite restrict cigarette smoking ban in indoor or some outdoor locations, the risk of non-smokers to be exposed to environmental cigarette smoke is not yet eliminated. Beside the well-known effects of cigarette smoke to the respiratory and cardiovascular systems, a growing literature has shown during the last 3 decades its noxious effects also on cutaneous tissues. Being the largest organ as well as the interface between the outer environment and the body, human skin acts as a natural shield which is continuously exposed to harmful exogenous agents. Thus, a prolonged and/or repetitive exposure to significant levels of toxic smoke pollutants may have detrimental effects on the cutaneous tissue by disrupting the epidermal barrier function and by exacerbating inflammatory skin disorders (i.e. psoriasis, atopic dermatitis). With the development of very complex skin tissue models and sophisticated cigarette smoke exposure systems it has become important to better understand the toxicity pathways induced by smoke pollutants in more realistic laboratory conditions to find solutions for counteracting their effects. This review provides an update on the skin models currently available to study cigarette smoke exposure and the known pathways involved in cutaneous toxicity. In addition, the article will briefly cover the inflammatory skin pathologies potentially induced and/or exacerbated by cigarette smoke exposure.

Keywords: *Cigarette smoke, skin models, inflammation, oxidative stress, psoriasis, atopic dermatitis*

Contents

Introduction

Environmental tobacco smoke (ETS), is defined as a mixture of the smoke obtained by burning tobacco products (cigarettes, cigars or pipes) and the smoke exhaled by smokers. It is a concentrated, complex and dynamic aerosol consisting of more than seven thousands of chemicals and is classified by the Environmental Protection Agency (EPA) as a Group A carcinogen.[1]

Despite policy-based interventions such as tax increases on tobacco, health care prevention campaigns, enforcing bans on advertising, cigarette consumption is still increasing in many countries and the epidemic is shifting towards the developing world.[2,3]

The cigarette smoke (CS) aerosol is produced by incomplete combustion during smoking and can be divided into two phases: a particulate and a gas phase. The particulate phase is the minor fraction and constitutes 4-9% of the total smoke by weight whereas the gas phase is the major fraction with 91-96%.[4]

The most harmful and carcinogen smoke toxicants identified are products of combustion which are found mostly in the gas phase, hence the importance of performing hazard and risk assessment using a combination of the particulate and gas phase (whole smoke) to better simulate "real life". In addition, the whole smoke considers interactions between the two phases that cannot be neglected. Examples of chemicals in the gas phase include aldehydes (formaldehyde, acrolein, 4-hydroxynonenal), hydrogen peroxide (H_2O_2), superoxide (O_2) and hydroxyl radical (OH), reactive nitrogen species (RNS) and hydrogen cyanide. Examples of chemicals in the particulate phase include polycyclic aromatic hydrocarbons (PAHs) and tobacco specific nitrosamines (TSNAs).

CS is associated with pulmonary[5,6] and cardio vascular diseases.[7,8] Many evidences have also shown detrimental effects of CS on skin. More specifically, the oxidative compounds derived from incomplete combustion of the cigarette can affect the cutaneous tissue.[9] Cigarette smoking has been linked to various dermatological conditions and pathologies: poor wound healing, squamous cell carcinoma, melanoma, acne, psoriasis, eczema, and hair loss.[10] Moreover, an epidemiologic study has shown that CS is one of the numerous factors contributing to premature skin aging, independent of age, sex, pigmentation, sun exposure history, alcohol consumption and other factors.[11] A study on twin pairs has demonstrated the obvious relationship between cigarette smoking and premature skin aging[12] and a British doctor, Douglas Model, defined the term "smoker's face" in 1985 to describe the wrinkling and premature aging associated with smoking.[13]

Beside affecting cellular redox homeostasis, CS components can provoke inflammatory skin responses. Numerous chemicals, especially PAHs, can penetrate the epidermal barrier and enter the systemic circulation through the capillaries in the dermis causing systemic effects.[14] These highly lipophilic compounds also readily penetrates skin cells. In this process the structural and functional integrity of the skin barrier is directly altered at the sites of CS interaction, or impaired through indirect pathways, such as the induction of pro-inflammatory mediators. Indeed, many skin disorders are related to a chronic inflammation response, for instance in both atopic dermatitis and psoriasis,[15] where the immune activation by release of cytokines influences keratinocyte proliferation and differentiation.

The use of an appropriate skin tissue model and CS exposure systems has become fundamental to study and identify the CS induced cutaneous toxicity pathways as realistic as possible. Understanding the mechanisms by which CS interacts with skin could support the development of therapeutic interventions.

The purpose of this review is to discuss the existing CS skin exposure approaches, and to provide an overview about relevant biomarkers studied to assess cutaneous toxicity to support the development of preventive and curative treatments.

I. Mimicking skin exposure to cigarette smoke

1. Skin models

Various skin models have been described to assess possible hazards and risks resulting from contact or exposure to chemical compounds. Nowadays, greater effort is put in the development of in vitro models based on human cells to replace animal testing. Indeed, in addition to have limited predictive capacity for human toxicity, they generate ethical issues. In vitro skin models are simple and promising tools for a wide field of applications such as cosmetology and regenerative medicine.

From the inside to the outside, skin strata start from the subcutaneous tissue, the dermis and finally the outermost layer, the epidermis. The epidermis consists in four or five layers of keratinocytes which synthesize protective proteins such as keratin and a lipidic matrix crucial for the skin barrier function.[16,17]

2D cell models

Cell lines are more homogenous populations in comparison to primary cells and show reduced donor to donor variability. Immortalized cell lines are readily available, stable, and easy to handle whereas primary cells have some passage number limitations. Therefore cell lines appear to be appropriate as an in vitro cell model for the rapid assessment of acute toxicity induced by pollutants.[18]

HaCaT cells, the first spontaneously immortalized human keratinocyte line exhibiting normal differentiation,[19] is the most used 2D skin model consisting mostly of keratinocytes.[20] Alternative cell lines exist such as A431, a human epidermoid carcinoma cell line,[21,22] and JB6, a mouse epidermal cell model. Both of them have been mainly used to study tumor promotion and progression.[23]

As fibroblasts are responsible for secreting substances of the extracellular matrix essential for keratinocytes growth, co-culturing keratinocytes with fibroblasts allows reproducing cellular crosstalk, especially of interest when studying wound healing mechanisms.[24,25] The main fibroblast cell line is known as the 3T3 cell line.[26]

Primary cell cultures are explanted directly from either healthy donors or subjects with pathologies and are considered more biologically relevant. Primary fibroblasts are an ideal model for studying skin aging in vitro. In comparison to primary keratinocytes that can only grow for about 7 passages, human primary fibroblasts can generally be grown in culture medium for up to 40-50 passages and become especially relevant for prolonged senescent studies.

Although easy, fast and inexpensive, a major shortcoming with 2D cell culture system is the sensitivity to pollutant exposure since it lacks the protective physiological cutaneous structure. Therefore, the use of a 3D skin model with a closer morphology to real skin combined with an exposure scenario is recommended to better understand some of the biological mechanisms linking pollution toxicity to skin structure disruptions.

3D skin models

Reconstructed human epidermis (RHE) is one of the most reliable in vitro models to investigate cutaneous stress responses. RHE closely mimics the morphological, biochemical and physiological properties of the human epidermis, and can be created in laboratories or commercially purchased.[27] 3D models allow the reproduction of exposure scenario closer to real-life situation, for instance by topically applying components on the epidermis. The main shortcomings of RHE are the poor barrier properties, the lack of vascularization, sweat glands and hair, the lack of representation of the physiologically-relevant desquamation process, and the lack of immune system even though several efforts have been put to create immune competent skin models with the integration of T cells.[28]

The 3D full-thickness (FT) skin model contains both dermal and epidermal parts separated by a basement membrane. A fully stratified keratinocyte-populated epidermis associated with a fibroblast-populated dermis permits intercellular signaling. Cell interactions between keratinocytes and dermal fibroblasts can affect the expression of proteins and is vital in skin homeostasis. Whereas such models are commercially available, they are less commonly used than reconstructed epidermis models, which are easier to handle, less complex and less costly.
To obtain an increased longevity of in vitro skin equivalents, adjustment and implementation into miniaturized conditions as found in chip technologies or into the environment of a perfused bioreactor is now feasible.[29,30,31]

In vivo models

The most common animal species used to date as skin models have been pigs mainly for the structural resemblance with human skin.[32,33] Nonetheless, pigs exhibit poor vascularization, a thicker skin epidermis and an increased amount of fat components compared to humans.[34]
Furthermore, animal testing has numerous drawbacks such as strict regulation, excessive costs, high variability (e.g. Local lymph node assay - LLNA)[35] as well as ethical concerns. Complying with the commitment of replacing animal testing in industrial toxicology laboratories, the validation of alternative in vitro test methods has become a crucial need. Today, non-invasive clinical methods involving small groups of human volunteers have also been used to evaluate the biological responses induced by cigarette smoke in contact with reduced skin areas. However, this controlled clinical method also generates a pollution stress on 'living skin'.[36]

Ex vivo skin tissue

Ex vivo human skin is usually obtained from plastic surgeries either directly from hospitals or distributed by tissue banks. For dermal absorption studies, skin explant is the preferred model since the barrier properties are well preserved after excision with controlled conditions. However, the donor dependency of skin explants is disadvantageous as it complicates the comparison of different studies. Restricted access and excessive costs are among limitations to the use of native human skin.[37,38,39]

In comparison to ex vivo skin tissue and in vivo testing, in vitro studies offer several advantages including: more flexibility, better reproducibility, investigation of cellular components and cost-effectiveness. In vitro models have however some limitations. It is well demonstrated that the permeation of exogenous agents through in vitro-based skin models overestimate the in vivo data,[40] as a consequence of a thinner stratum corneum and a less complex structure missing hair follicles, sebaceous glands and sweat glands. Culturing mammalian cells outside of their physiological environment, i.e. in an environment lacking protective and detoxifying element such as the microbiota, skin metabolism and the immune system, is also a drawback of the in vitro models and makes in vitro cultures more sensitive and vulnerable than in vivo / ex vivo models.

In conclusion it can be stated that there is no perfect model to study the cutaneous responses to exogenous challenges such as CS, and that new insights with regard to the toxicity pathways induced by CS must be obtained using different experimental approaches from 2D cell culture to 3D skin models.

2. CS exposure from life to bench

In the field of CS toxicity testing, many studies have developed relevant and appropriate cigarette smoke exposure methods trying to mimic real life conditions (see **Figure 1**). However, the lack of important information such as types of cigarette, exposure time, smoking machine characteristics etc. makes it difficult to compare among different studies.

Cigarette type

Whereas some researchers have used commercial cigarettes for their studies,[41,42] the typical cigarettes used for experimental purposes are "reference 3R4F cigarettes" which have a standardized composition.[43] The cutaneous toxicity of those cigarettes is usually evaluated by exposing a skin model to the smoke of a cigarette either extracted into a culture medium or delivered into an exposure chamber.

Cigarette smoke extract (CSE)

Cigarette smoke extract (CSE) is collected via a trapping system and is then usually dissolved in cell culture medium or buffer. Typically, CSE is prepared at a concentration of 1 cigarette/25mL in serum-free cell culture medium.[44,45,46] The resulting solution which is always at saturation is defined as the highest concentration (100% CSE) and is then diluted to various concentrations. CSE is used after adjusting the dispersion to pH 7.4 using sodium bicarbonate and filtering through a 0.22 µm filter.[22,23] Depending on the solvent used, CSE composition can vary. It can be either aqueous using PBS[48] or organic using hexane. An organic solvent is mainly used to collect a large amount of PAHs which are slightly soluble in water.[49] Although this exposure method is simple and quick for in vitro studies, there is a lack in physiological relevance to mimic the real human skin exposure. The main weakness of CSE relies on its composition as it mainly contains the particulate phase and excludes the volatile compounds such as aldehydes (acrolein and acetaldehyde) that are major contributors of CS induced toxicity.[50] In addition, CSE solution should be prepared immediately before being applied to avoid any aging effect of the extract. Fresh CSE is very different from aged CSE due to the presence of short-lived reactive species that are no longer present in old CSE.[51,52,53] The cigarette smoke extract has been used to study the effects of cigarette smoke on skin pathology in vivo and in vitro. For instance, in fibroblasts, approximately 20% of the cells survived in 100% CSE, and at 10% CSE approximately 80% of the cells survived.[44] Chronic effects of CS on skin can be simulated by dosing CSE concentrations, for instance using a low concentration for 20 days to minimize toxicity.[54]

Cigarette smoke chamber (CSC)

Cigarette smoke can be simply generated using small devices such a vacuum pump or an electrical fan. However, to achieve a better repeatability and reproducibility in terms of smoke composition and volume, it is recommended to use specific smoking machines that generate smoke at a standardized rate. Those smoking machines are often connected to an exposure chamber where small fans provide a homogeneous distribution of the smoke over the skin models under investigation. Several smoking machines with different settings and designs are commercially available.[55,56,57,58]

The use of smoking machines allows to expose the model of interest to the so called ETS (environmental tobacco smoke) which includes on the one hand both the mainstream and second-hand smoke, and on the other hand both the particulate and gas phase. Furthermore, it is possible to filter the particulate phase at the opening of the exposure chamber to expose the skin models to the gas phase only. By providing a better quantification of the exposure dose (time, airflow, ratio aerosol/air, puff number,

yield of delivered smoke), these smoking systems will be particularly useful for cross-platform comparisons.

To conclude, these well-developed exposure chamber systems address the need for a standardized and reproducible method for skin exposure to CS. **Tables 1 and 2** summarize the skin models used over time to assess the adverse effects of CS exposure on skin using the two main routes of exposure set up in laboratories: CSE (**Table 1**) and CSC (**Table 2**).

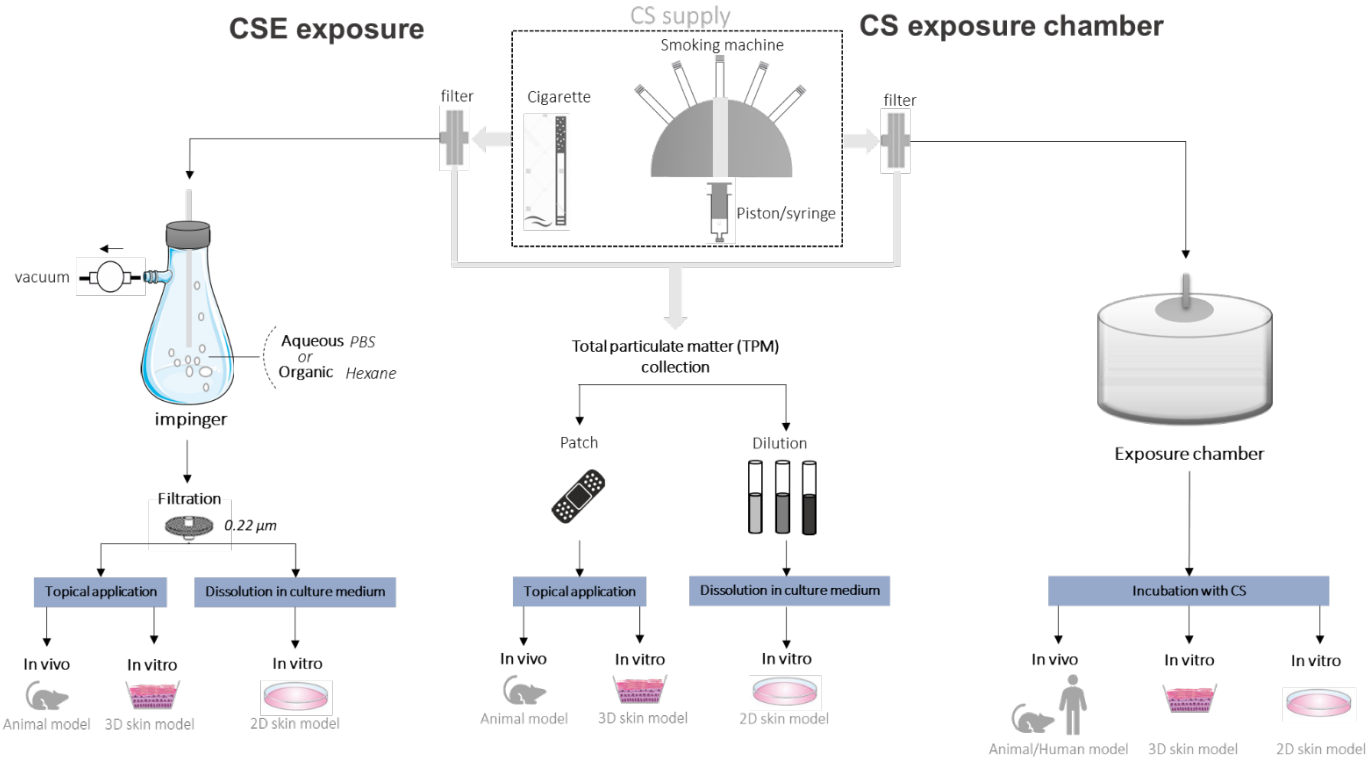

Figure 1: Overview of predominant CS routes of exposure: Cigarette Smoke Extract (CSE) and Cigarette Smoke Chamber (CSC)

2-column fitting image

Table 1: Types of skin models exposed to cigarette smoke extract (CSE) to investigate the cutaneous toxicity induced by CS

Model type	*Model description*	*References*
In vitro (2D cell model)	Human keratinocyte cell line (HaCaT)	[59] [60]
	Murine keratinocyte cell line (PAM212)	[61]
	Normal human epidermal keratinocytes (NHEK)	[54]
	Normal human dermal fibroblasts (NHDF)	[48] [62] [44] [63]
	Normal human gingival fibroblasts (NHGF)	[50]
	Normal human epidermal melanocytes (NHEM)	[64]
In vitro (3D cell model)	Reconstituted human epidermis model derived from primary keratinocytes (StratiCELL RHE/001 model)	[65]
In vivo	Mice (SENCAR)	[66]
	Chicken	[67]
Ex vivo	Rat	[68]

Table 2: Types of skin models exposed to cigarette smoke chamber (CSC) to investigate the cutaneous toxicity induced by CS

Model type	*Model description*	*References*
In vitro (2D cell model)	Human keratinocyte cell line (HaCaT)	[69] [70] [71] [72] [73]
	Normal human dermal fibroblasts (NHDF)	[74]
	Coculture of HaCaT cell line and human fibroblast cell line (HFF-1)	[75]
	Coculture of HaCaT cell line and human sebocytes cell line (SZ95)	[45]
In vitro (3D cell model)	Reconstituted human epidermis (RHE) model derived from primary keratinocytes (SkinEthic™ model)	[41]
	Reconstituted human epidermis (RHE) model derived from primary keratinocytes (EpiDerm™ model)	[76]
	Full-thickness (FT) skin model derived from NIKS cells seeded onto dermal equivalents comprised of normal human dermal fibroblasts embedded in type I collagen (StrataTest® model)	[77]
In vivo	Mice (C57BL/6)	[78] [79]
	Mice (SKH1)	[80]
	Rats	[81]
Ex vivo	Human full-thickness skin	[74]

E-cigarette

Electronic cigarettes have become extremely popular in a short period of time, being presented as a safe alternative to traditional cigarettes. While the toxins from cigarette smoke are caused primarily from burning the tar, e-cigarettes generally contain cartridges filled with nicotine and other chemicals turning

into a vapor or steam that is inhaled by the smoker. Although more research needs to be done in order to truly determine the effect of e-cigarettes on individual health, there seems to be some evidence that points to its harmful effects on health.[82,83] A recent case in the US has accounted the fifth patient death of lung disfunction after significant e-cigarette consumption.[84] In fact, e-cigarettes contain harmful substances as well, including propylene glycol, glycerol, a variety of flavoring substances[85] and a similar dose of nicotine to cigarettes known to delay wound healing and accelerate skin aging.[86] A review of studies found that levels of toxins and metals in e-cigarette aerosol varied considerably within and between brands.[87] In addition, previous work have demonstrated that the flavoring substances from e-cigarettes were the most toxic[88] in embryonal and adult cells and in epithelial cells such as HaCaT cells.[69,89] Studies have also shown that the only mechanical action of puckering your lips causes deep lines and wrinkles around the mouth.[90]

Despite a number of studies introducing e-cigarettes as safer alternative to traditional cigarettes because of their lack of tobacco, tar and combustion,[91] they have still not proven to be safer and have not been approved by FDA as a cessation aid.[92] Since vaporizers are a new technology, the long-term effects are still unknown.

II. Cigarette smoke induces cutaneous toxicity

CS exposure is known to increase pro-inflammatory mediators and oxidative stress markers in many organs such as lung, cardiovascular, intestine and skin. Skin is one of the most direct targets of CS exposure and many studies have shown the ability of CS to affect skin homeostasis. Oxidants contained in CS induce adverse effects on the cutaneous tissue through oxidative modifications of key biological structures as well as inflammatory responses.

1. CS induces an inflammation response

Inflammation is a tissue response to damage that involves a sequence of activated cells able to secrete inflammatory mediators like cytokines and chemokines.[93] Overproduction of pro-inflammatory cytokines such as interleukins IL-1α, IL-6 and IL-18 have been widely used as inflammatory biomarkers for assessing the adverse effects of CS in skin models.[41,69,75,80,94]

Tumor necrosis factor-α (TNF-α) is another cell-signaling cytokine often found in studies investigating the noxious effects of CS. TNF-α is involved in disease pathologies such as psoriasis, contact dermatitis, drug eruptions, and cutaneous T-cell lymphoma.[95,96,97] TNF-α regulates gene expression in response to environmental damage and induces inflammation both locally and systemically. Low level of TNF-α is present in the upper layer of the healthy epidermis, but its synthesis and release from keratinocytes are significantly increased after exposure to CS as shown in several studies performed with HaCaT cell lines[46,69] and mice skin tissues.[98,99] Thus, low levels of TNF-α are essential to perform key homeostatic functions but an overproduction of TNF-α and other pro-inflammatory markers weakens the host defense against pollution and contributes to the development of inflammatory skin diseases.

IL-8 represents one of the major cytokines involved in inflammatory response. This chemokine mediates the recruitment and activation of neutrophils via signaling mechanisms and extracellular adhesion molecules.[100] The pathogenetic role of IL-8 has been suggested in inflammation-related skin diseases such as psoriasis,[101] palmoplantar pustulosis,[102] and acne where *Propionibacterium acnes* induces its production in various cell types including primary keratinocytes.[103,104] The secretion of IL-8 is stimulated when skin is exposed to environmental stressors such as CS as shown by several studies on primary keratinocytes[41] and reconstructed human epidermis.[105] **Table 3** gives an overview about the inflammatory biomarkers used to assess the cutaneous responses to CS exposure in vitro and in vivo.

Table 3: Review of inflammatory biomarkers reported in studies assessing the cutaneous toxicity induced by CS

Model type	*Cell type*	*Exposure condition*	*Biomarkers*	*Main observations*	*Reference (Year)*
In vitro (2D cell model)	HaCaT	CSE	EGR-1, MAPKs, TNF-α, p-ERK1/2, p38 kinase, p-JNK1/2	Increase of EGR-1 protein and TNF-α in a dose-dependent manner Increase of p-ERK1⁄2, p-JNK1⁄2 and p38 kinase in a time-dependent manner	[46] (2010)
		CSC	NF-κB-aldehydes adducts formation	Drastic increase of Acrolein (ACR) Increase of 4HNE-p65 and ACR-p65 adducts	[71] (2010)
			Various cytokines	Increased release of IL-1α, IL-6, IL-10, G-CSF, IFN-c, RANTES, TNF-α and VEGF	[69] (2014)
			IL-1α, IL-8	Increased release of IL-1α and IL-8	[75] (2017)
	PAM212	CSE	NF-κB, TSLP, p-IKβ-α, IKβ-α, p-AMPK, p-ERK1/2	Suppression of NF-κB activation through the α7 nAChR–PI3K–AMPK signaling pathway. Decreased release of TSLP	[61] (2016)
	NHDF	CSE	TβR-II, p-ERK1/2, EGR-1	Decreased expression of TβR-II mRNA Increase of p-ERK1⁄2 in a time-dependent manner Increase of EGR-1 in time- and dose-dependent manners	[63] (2010)
			EGR-1	Increase of EGR-1 in a time-dependent manner	[44] (2011)
	NHEM	CSE	AhR, MITF, β-catenin	Increased activation of AhR Increased expression of MITF and β-catenin in a dose-dependent manner	[64] (2013)
In vitro (3D cell model)	RHE	CSC	IL-1α, IL-8, IL-18	Increased release of IL-1a, IL-8, and IL-18	[41] (2016)
In vivo	SKH1 mice	CSC	IL-6 and IL-8 p-ERK1/2, p22phox, p47phox, p66Shc	Increase of IL-6 and IL-8, p-ERK1/2, p22phox, p47phox, and p66Shc	[80] (2012)
	C57BL/6	CSC	Myeloperoxidase (MPO) TNF-α, IL-1β, IL-6, NF-κB p65 subunit	Increased activity of MPO Increased release of TNF-α, IL-1β, IL-6 Increased expression of NF-κB p65 subunit	[99] (2016)

2. CS alters redox homeostasis

Skin redox balance is an equilibrium between the generation and the scavenging of reactive oxygen species (ROS). ROS can derive from exogenous sources such as CS as well as from endogenous sources such as NADPH oxidase, xanthine oxidase, and myeloperoxidase (MPO). Failure to maintain the physiological redox steady state is defined as oxidative stress[106] and has been linked to premature skin aging,[107] chronic inflammation and skin diseases.[108]

As demonstrated in many studies, upon exposure to CS oxidant species an increase of intracellular ROS[42,47,77] and protein carbonyls[42,76,80] levels has been observed in cutaneous tissues, in vitro and in vivo. This leads to the generation of reactive electrophilic molecules, such as reactive aldehydes - e.g. 4-hydroxy-2-nonenal (4-HNE)[41,45,80] and malondialdehyde (MDA)[68,99,109] - that can induce irreversible damage.[110] Due to the close interaction between lipids and proteins in tissues, these lipid peroxidation products can cause protein-adduct formation and crosslinking, progressively leading to impaired protein function and ultimately to cell dysfunction, inflammatory response and apoptosis.[111] An increase in lipid peroxidation correlates as well with a diminution of the skin antioxidant capacity as both antioxidant compounds (e.g. vitamin C, vitamin E, uric acid, and glutathione aka GSH) and enzymes, e.g. superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) are depleted or inactivated after exposure to environmental stressors such as CS.[42,47,54] Cytoprotective enzyme heme oxygenase (HO-1), which plays an important role in cellular protection and oxidative stress reduction, has been shown to be rapidly upregulated in response to CS in skin[80] and lung models.[112] **Table 4** lists all the predominant biomarkers of oxidative stress studied to assess CS toxicity in vitro and in vivo.

Table 4: Review of oxidative stress biomarkers reported in studies assessing the cutaneous toxicity induced by CS

Model type	*Cell type*	*Exposure condition*	*Biomarkers*	*Main observations*	*Reference (Year)*
In vitro (2D cell model)	HaCaT	CSC	GSH, ROS, protein carbonyls	Increase of protein carbonyls in a dose-dependent manner Increased levels of GSH and ROS	[42] (2014)
	HaCaT + SZ95	CSC	4-HNE	Increased levels of 4-HNE/SRB1 adducts	[45] (2017)
	NHDF	CSE	GSH-Px, SOD, ROS	Decrease of GSH-Px and SOD activity Increased levels of ROS	[47] (2013)
	NHEK	CSE	SOD	Decrease of SOD activity	[54] (2016)
In vitro (3D cell model)	RHE	CSC	4-HNE	Increased levels of 4-HNE	[41] (2016)
			Protein carbonyls	Increased levels of protein carbonyls	[76] (2017)
	FT	CSC	ROS	Increased levels of intracellular ROS	[77] (2010)
In vivo	SKH1 mice	CSC	4-HNE, HO-1 Protein carbonlys	Increased levels of 4-HNE, HO-1 and protein carbonlys, more importantly in young (vs. old) mice	[80] (2012)

3. Cross talk inflammation and oxidative stress in skin

Exogenous factors like CS are able to affect cutaneous homeostasis by inducing a pro-inflammatory status and modifying tissue redox homeostasis.[106,113] Failure to control oxidative stress can stimulate the skin to possibly develop inflammatory-related skin conditions.[93] CS exposure activates a series of transcription factors, such as aryl hydrocarbon receptor (AhR), nuclear factor kappa-B (NF-κB) and activator protein-1 (AP-1) that, in turn, can induce a combination of inflammatory mediators and contribute to an immune dysfunction and an altered cytokine profile.

The AhR is a cytosolic ligand-activated transcription factor found in various types of skin cells and widely expressed in the skin response to external environmental signals.[114,115,116] It plays a crucial role in skin detoxification and inflammation regulation[117,118] but is also involved in epidermal differentiation and attachment.[118] After exposure to CS, an inflammatory cascade of cytokines and chemokines has been observed following AhR activation in vitro[59] and in vivo.[99] Xenobiotic molecules such as PAHs, contained in CS, transdermally permeate skin and form a complex with AhR.[119] Activation of AhR results in its translocation from the cytosol to the nucleus, where it forms a heterodimer with the AhR nuclear translocator (ARNT) and binds to specific DNA consensus sites known as xenobiotic response element (XRE). DNA binding of AhR in keratinocytes induces the generation of cytochrome P450 1A1 (CYP1A1) and ROS, the production of 8-hydroxydeoxyguanosine (8-OHdG), a well-known DNA damage marker, and inflammatory cytokines.[105]

NF-κB redox sensitive transcription factor has been reported to be the common pathway for the conversion of environmental insults into inflammation in the skin. Perturbations in its activity such as overactivation[120] or inhibition[121,122] are linked to the development of skin defects, inflammatory skin disease, and skin cancer.[123] NF-κB acts in immune and non-immune cells to control the maintenance of tissue immune homeostasis which is maintained by an extensive cross-talk between epidermal keratinocytes and immune cells.[124] NF-κB inhibition disturbs the response of the epidermis to environmental challenges and compromises the communication between the epidermis and the dermis, triggering an inflammatory response that resembles a wound-healing reaction.[125,126] Exposure to CS have been shown to induce NF-κB activation in keratinocytes via the activation of endogenous sources of ROS.[71]

It has been demonstrated that the activation of the NADPH oxidases (NOX) is involved in both migration and proliferation in extensive cell types such as epithelial cells, fibroblasts, and vascular endothelial cells therefore playing a critical role in skin physiology.[127] NOX also contributes to the pathogenesis associated with impaired immune responses due to environmental factors.[128] Once activated by exogenous stimulus, cytoplasmic NOX components, p67 phox and p47 phox, translocate to the membrane to form the NOX complex (NOX2) and induce H_2O_2 production via the generation of superoxide (O2–).[70]
In addition, ERK1/2 activity in keratinocytes takes part to a homeostatic mechanism regulating inflammatory responses[129] and its phosphorylation is stimulated by CS in fibroblasts,[63] keratinocytes[46] and mice skin.[80]

In the imbalanced state of oxidative stress, specific transcription factors (i.e. AP-1 and nuclear factor erythroid-2-related factor 2 aka Nrf2) are known to be activated.[130,131,132] The AP-1 transcription factor, mainly composed of Jun and Fos protein dimers, is a key regulator of epidermal keratinocyte survival and differentiation and important driver of cancer development.[133] The Nrf2 transcription factor plays an essential role in maintaining skin redox balance by regulating numerous genes involved in the defense against environmental stressors. Nrf2 activation promotes repair of a deficient epidermis and its

activation has detrimental effects on skin, therefore its dysfunction can be associated with various human skin diseases.[131] Although mostly demonstrated on lung models, both AP-1 and Nrf2 transcription factors are stimulated after CS exposure.[134,135]

III. Cigarette smoke acts as a potential trigger of inflammatory skin pathologies

As seen in the previous sections, CS exposure imbalances skin homeostasis through generation of oxidative damage and inflammatory responses leading to the pathogenesis of several skin disorders. Although the exact mechanism is not clearly identified because of a large variety of toxins in cigarettes, CS exposure stands as a potential environmental risk factor for the development or exacerbation of inflammatory skin pathologies. **Figure 2** illustrates the general mechanism of CS as a risk factor for the development or aggravation of inflammatory skin conditions such as psoriasis and atopic dermatitis.

1. Psoriasis

Psoriasis is a multifactorial inflammatory skin disease affecting 3% of the population.[136] The most common form is chronic plaque psoriasis, accounting for 90% of cases and presenting with monomorphic lesions that are erythematous and scaly. In the pathogenesis of psoriasis, there is an interplay between immune cells and keratinocytes.[137] Psoriasis skin pathology is mainly associated with excessive secretion of inflammatory cytokines by T-cell populations, infiltration of neutrophils and T cells[138] as well as hyperactivation of the transcription factor STAT3 (Signal Transducer and Activator of Transcription 3), which is associated with hyperplasia.[139] Cells of the innate immune system such as dendritic cells and macrophages facilitate the differentiation of Th17 cells through production of IL-23 and IL-6, leading to a Th17-skewed adaptive immunity. Consequently, Th17 cells secrete IL-17A and IL-22 that stimulate epidermal keratinocytes which in turn produce pro-inflammatory cytokines (e.g. IL-1, IL-6, and TNF-α) and chemokines (e.g. IL-8 aka CXCL8, interferon-inducible protein 10 aka CXCL10, and macrophage inflammatory protein-3 alpha aka CCL20) that will further interact with the innate immune system. This crosstalk results in an inflammatory loop or "vicious circle" involving resident epidermal cells, as well as innate and adaptive immune cells.[140]

In respect to the abnormal epidermal barrier formation, the activation of keratinocytes leads to an excessive and atypical epidermal differentiation and proliferation (hyperkeratosis and parakeratosis), neutrophil influx into the epidermal compartment, production of antimicrobial peptides (AMPs) including LL-37, β-defensins, and psoriasin (S100A7) as well as a deficient synthesis of intercellular lipids in the extracellular space.[141,142] In addition, in psoriatic skin, the components of the stratum corneum (SC) are prematurely synthesized in the stratum spinosum and the expression of the early differentiation markers such as involucrin (INV) and corneodesmosin (CDSN) is upregulated, while the expression of the late differentiation markers like loricrin (LOR) and filaggrin (FLG) is downregulated.[143] These constituting proteins covalently linked together by transglutaminases and located on the inside of the plasma membrane of terminally differentiated keratinocytes, called corneocytes, form the so-called cornified envelope and have an important protective role against trauma, ultraviolet irradiation, and infections.[144] An altered formation of the cornified envelope, as in the case of psoriatic lesions, significantly affects the barrier capacity to retain water leading to the formation of scales or flakes arising from the shedding of SC fragments.[145,146] Keratinocytes of psoriatic skin have an epidermal turnover of 6-8 days compared to approximately 45 days in normal skin.[147] In psoriasis lesions, the SC is thicker and disorganized, the granular layer is almost inexistent and the hyper-proliferative basal layer appears as finger like projection known as the dermal papillae.

Besides a genetic predisposition and its autoimmunity, psoriasis can also be exacerbated or triggered by environmental factors such as traumatic injury to skin, physical and psychological stress, cold weather, excessive alcohol and drugs intake,[148] nutrition[149] and smoking.[150,151] The altered physical barrier and weakened defense mechanism make psoriatic skin more vulnerable to external aggressors such as CS.

Ozden et *al.* evaluated risk factors associated with the development of pediatric psoriasis and observed that maternal and environmental tobacco smoke exposure may influence the development of pediatric psoriasis.[152] Although mostly demonstrated in lung models,[153,154,155,156] CS plays a role in the exacerbation of psoriasis by enhancing the differentiation of Th17 cells,[157] which play a predominant role in pathogenesis and production of pro-inflammatory cytokines.[46,158]

2. Atopic dermatitis

Atopic dermatitis (AD) is an intensely pruritic, chronic, inflammatory skin disease affecting 10–20% of children and 3–5% of adults, therefore representing a growing health concern. AD is clinically characterized by intense pruritus and erythema and, in its chronic form, by thin scaling, focal parakeratosis and lichenification (i.e. thickening of the epidermis).[159] Similarly to psoriasis, the cause of AD is multifactorial, with genetic[160] and environmental factors.[161,162] Both diseases share common pathogenetic processes involving an impaired immune response, a T-cell mediated skin barrier function deficiency and a similar STAT3 hyperactivation involved in hyperplasia. However, AD epidermal disruption is not only driven by the secretion of IL-17, IL-22 and interferon gamma (IFNγ) proteins which are produced by Th17, Th22 and Th1 cells, respectively. It is also induced by the secretion of IL-4 and IL-13 following Th2 activation.[15] These two cytokines are able to suppress FLG expression resulting in a reduced keratinocyte terminal differentiation and epidermal barrier defect.[163] Recently, loss-of-function mutations in the FLG gene have been identified in AD patients, therefore suggesting skin barrier deficiency as a major cause of the disease.[164,165,166] As a consequence, the prevalence of null allele for the FLG gene has been correlated with a decreased levels of natural moisturizing factor (NMF) molecules and a dry skin phenotype.[167,168] AD is also characterized by an overproduction of immunoglobulin E (IgE) by B-cells,[169] allergies and asthma-associated features as well as a distinguishable overexpression of the thymic stromal lymphopoietin (TSLP) receptor.[170] The role of TSLP was found to induce itch by acting directly on nerve fibers.[171] Itching and pruritus provoke in turn scratching and hence aggravate barrier disruption. This vicious cycle is called the "itch–scratch cycle".[172] Chronic itching and subsequent dysbiosis with predominant colonization by Staphylococcus aureus contribute to the maintenance of AD.[173] AD affects differently the populations according to their racial skin type; for instance, the disease is more prevalent in Black and mixed-race individuals compared to Whites.[174] Furthermore, Asian AD demonstrates closer patterns to psoriasis than European-American such as more epidermal acanthosis, neutrophil infiltration, and co-expression of IL-17 and IFNγ.[15,175]

An incomplete skin barrier repair after an injury might facilitate the penetration of environmental antigens or stimuli and as result trigger an enhanced inflammatory process. Exposure to CS has been clearly associated with AD symptoms,[176] acting as risk factors for its development or aggravation. Indeed, AD was significantly associated with active and passive smoking in Korean adolescents and in children and adults and even dogs according to recent studies.[177,178,179] A study involving 7030 children aged between 6 to 13 years demonstrated a positive correlation between AD and maternal smoking during pregnancy and/or in the first year after birth.[180] Prenatal exposure to CS is likely to be linked to the development of AD through immune dysregulation.[162]

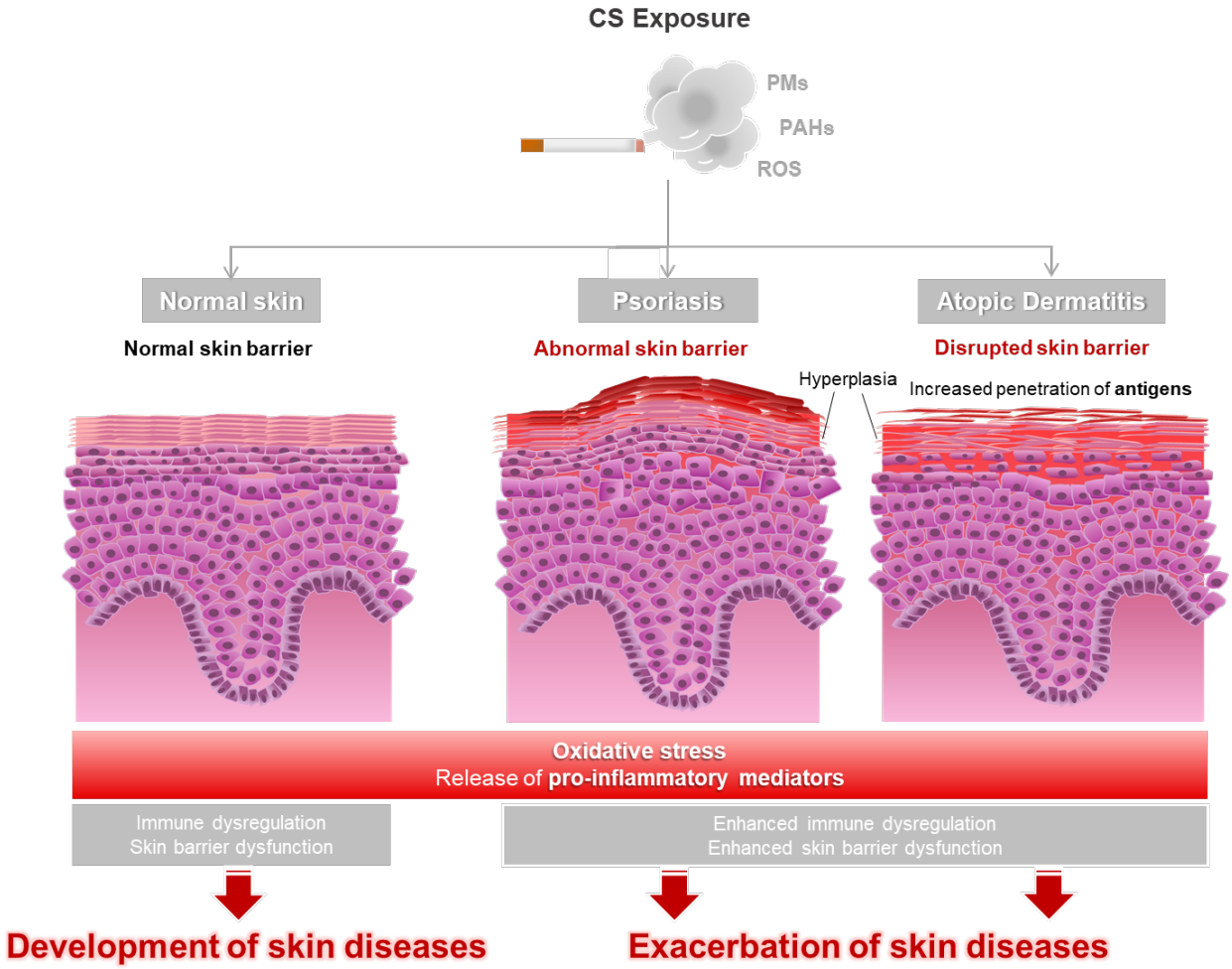

Figure 2: CS acts as a potential environmental trigger of oxidative stress and inflammation in normal, psoriatic and AD skin, impacting negatively the skin barrier function.

PMs: Particulate Matters; PAHs: Polycyclic Aromatic Hydrocarbons; ROS: Reactive Oxygen Species

2-column fitting image

3. Other skin disorders

Vitiligo is a depigmenting disorder in which epidermal melanocytes are destroyed. While the pathogenesis of vitiligo remains unclear, oxidative stress has been considered to be one predominant factor in the initiation of the disease through melanocyte destruction caused by ROS accumulation.[181,182] In vitiligo, melanocytes are particularly vulnerable to oxidative stress due to the pro-oxidant state generated during melanin synthesis and to the genetic antioxidant defects. This could suggest a potential role of CS exposure as a trigger of the disease.[183]

Acne vulgaris, a chronic inflammatory disorder involving sebaceous glands, ducts, and hair follicles, is caused by a combination of genetic, environmental, and hormonal factors. Acne affects mostly children and adolescents, and is clinically characterized by comedones, papules, pustules, and cysts.[184] CS is a potential environmental risk factor in the development or exacerbation of acne vulgaris. Epidemiological studies on the German population by Schäfer et al. showed that smoking was a clinically important contributory factor to acne prevalence and severity.[185] In addition, Capitanio et al. reported a positive correlation between cigarette smoking habits and adult women with acne. Nicotine, an agonist of acetylcholine (ACh) is suspected of inducing hyperkeratinization and comedogenesis via the stimulation of nicotinic acetylcholine receptors (nAChRs) on epidermal keratinocytes.[186]

CS exposure impacts the closure of cutaneous wounds via upregulation of genes involved in cell migration and downregulation of genes involved in inflammatory and immune responses, as demonstrated in vivo in dorsum excisional wound assay.[67,187] Nicotine was shown to promote wound healing,[188,189] to induce angiogenesis,[190] and to increase cutaneous blood flow by activating nAChRs.[191] Obviously, the risk of developing poor wound healing properties is not only limited to smokers but also to the non-smokers exposed to secondhand smoke.[74,81]

Finally, CS has been associated with increased incidence and production of basal and squamous cell carcinomas. Due to its high content in PAHs, CS may cause DNA damage, DNA repair system damage, as well as an upregulation of cell proliferation via AhR binding activation, generation of ROS and pro-inflammatory cytokines.[192]

Conclusion

CS exposure has been clearly associated with cutaneous toxicity by its ability to induce oxidative damage and inflammatory responses disrupting the skin barrier function which can lead to the development or exacerbation of inflammatory skin diseases, premature skin aging, and possibly skin cancer. The challenge remains to counteract its deleterious effect on skin by identifying the related inflammation pathways and associated biomarkers that could provide guidance towards a preventive or a curative treatment such as topical anti-inflammatory technologies, receptor antagonists, and barrier enhancers. For this purpose, researchers require the use of a relevant skin model and appropriate exposure testing methods to fully understand the impact of CS on skin. First, it is necessary to establish a standardization of exposure conditions to be able to evaluate the protective or preventive effects of dermo-cosmetic solutions against environmental pollutants since most research laboratories cannot afford the high costs of commercial exposure chamber systems. Secondly, selecting the optimal skin tissue model varies depending on the research application. Although animal models remain essential for risk analysis, alternatives should be used whenever non-animal-based experimental set up with similar relevance are available. Being comparable and consistent, in vitro skin models, especially the 3D reconstituted models, offer a potential for a wide range of applications. Thus, combining results from normal primary human cells and reconstituted human tissue cultures with data from human epidemiology and clinical studies might help to better extrapolate lab results to real life conditions.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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